# MBS-HACCP&ACQUE EASY TEST

## *BASIC SYSTEM*

# MBS MULTI-READER

## *USER MANUAL*

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## 1.1 Introduction

Dear User, thank you for purchasing **MBS-HACCP&ACQUE EASY TEST**, an innovative rapid colorimetric system to perform microbiological tests on food, water and surfaces, developed in collaboration with Roma Tre University.

The method of analysis is based on the observation of the color change in the suspension formed in the analysis vial used when the test sample is added: the suspension changes color (turns) if there are microorganisms, the greater the amount of microorganisms, the more rapid is the change of color.

The main features of the **MBS-HACCP&ACQUE EASY TEST** are:

- **Speed**: analysis time, from preparation to the achievement of results, from 2 to 5 times less than traditional methods;
- **Ease of use**: Anyone, anywhere can do the analysis without the need for other reagents or special equipment;
- **Sensitivity**: you can detect even a single microorganism present in the sample;
- **Selectivity**: it can detect different species of microbial organisms to the experimental limit of 99.999%;
- **Cost**: The cost of each analysis turns out to be 2 to 4 times cheaper than traditional methods.

The MBS method has been validated according to ISO 16140:2003 "Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods".

Available reagents for the selective search of the following microorganisms:

1. Total Viable Count – CBT-A01;
2. Coliforms (Totals ed *E. coli*) – CO-A02;
3. *Enterobacteriaceae* – EB-A03;
4. *Staphylococcus aureus* – SP-A04;
5. *Pseudomonas aeruginosa* – PAO-A05;
6. *Salmonella* spp. – SL-A06;
7. *Listeria* spp. – LY-A07;
8. *Enterococcus faecalis* – EF-A09;
9. Molds (*Aspergillus* spp.) – AS-A10;
10. Yeasts (*Saccharomyces* spp.) – SC-A11;
11. *Clostridium perfringens* – CP-A17.

## 1.2 How to use

The kit comes in a pack containing all the material for the analysis: the reaction vial (vial) and a vial of distilled water (vial of water).

To perform the analysis you must also have a bacteriology thermostatic incubator programmable to 30 °, 37 ° or 44 °C.

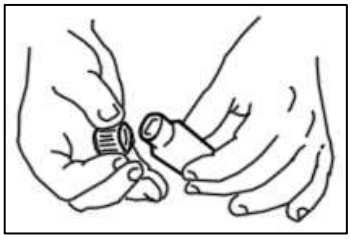
**Before handling the vials and proceeding with the analysis a thorough hand washing is recommended.**

The performance analysis can be summarized into 5 phases: opening, insertion of the sample, early analysis, order analysis and sterilization. It also differs by type of sample to be analyzed: **solid sample**, **liquid sample**, or **surface analysis**.

### 1.2.1 Solid sample

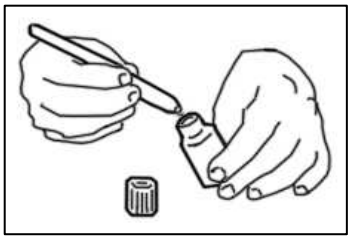
**Step 1. Opening**

- Open the vial, taking care to flip the cap so that the inner surface does not come into contact with the surface to avoid contamination.



**Step 2. Inserting the sample**

- Take a small aliquot of the food (no bigger than a grain of maize, corresponding to approximately 1 g) with a tool used during the processing of the food itself and insert it into the vial (alternatively you can use sterile tweezers).



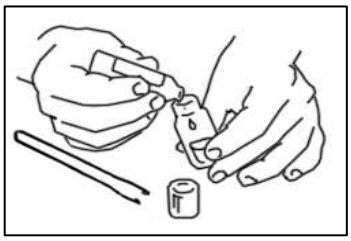
***Note: the size or the exact weight of the sample to be examined is not important.***

*Infact, the statistical variability microbiological examination of food samples is such that no method of analysis (including the reference method) has a variability of less than ± 60-70%. Therefore, the analysis has the same intrinsic value if it fits into a 1 g vial 2 g or 0.5 g. This variability is due both to the method of analysis, and to the sample itself since these microorganisms often grow in grouped colonies and therefore are not dispersed evenly in the middle.*

***Note: for inserting the sample into the vial**, we recommend using a tool used during the processing of the food itself, since by so doing, you will be able to detect any contamination of the food due to extrinsic causes.*

**Step 3. Start analysis**

- Open the vial of water supplied with the reaction vial, and insert the entire contents of the vial itself.
- Mix by inverting the vial several times to completely



dissolve the reagent (twenty seconds).

- If necessary, fill in the Quality Control Sheet.
- Place the vial in the incubator thermostat.

***Note:*** *the vial grows 2 liquid phases: the upper one consisting of white transparent oil (does not change during the analysis), formed at the bottom of the vial from the water with which the reagent is dissolved. The color of the second phase will develop fully within 15-20 minutes from the dissolution of the reagent and that is what must be observed for the evaluation of the results of the analysis.*

**Step 4. Control of the analysis results**

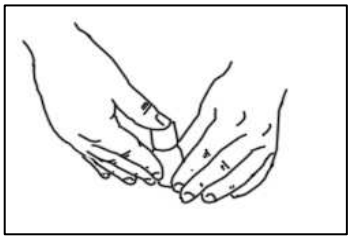
- You only need to check the color of the vial once, after a set number of hours depending on the type of analysis. This number of hours does not depend only on the type of analysis, but includes the operational limit of acceptable bacterial load in the sample (the generally accepted limits for the type of sample taken by law, are shown, **by way of illustration** on the Quality Control Sheets, ,).
- Compare the color of the vial with the correlation table set out in paragraph 1.3 or in Quality Control Sheet.
- If you have started the compilation of the Quality Control Sheet, fill it in with the result of the analysis.
- In the case of analysis for detection of *E. coli* and **only if the result is positive** (bright yellow vial), you can perform a test to confirm the presence of *E. coli*, adding 3-5 drops of Kovacs reagent to the vial at the end of the analysis, **before the sterilization**. If there is *E. coli*, within a few seconds you will see the development of a small surface layer of purple-red liquid.

***Note: to make the test for confirmation of E. coli, open the vial carefully****, avoiding contamination with the liquid contained in it, because if the color is tacked, the vial will contain a significant number of bacteria and it is therefore preferable to use disposable gloves, even non-sterile, for the operation of opening the vial. If you come in contact*

*with the liquid, wash the affected area immediately and thoroughly with antibacterial soap or normal soap and then with a disinfectant.*

**Step 5. Post-analysis sterilization**

- Without opening the vial, firmly press the top of the cap and shake for about 10 seconds. The addition of the sterilizing agent can cause a color change. After 5-10 minutes the contents of the vial are completely sterilized.

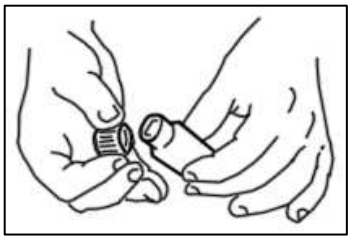


***Note:*** *when the sterilization of the microorganisms are destroyed, the reagents are rendered inert and the vial can be safely disposed of as a "sanitary waste non-hazardous" according to the D.M. of 25/5/89. Such waste, after sterilization, can be disposed of in the same way as drugs which have expired.*

### 1.2.2 Liquid sample

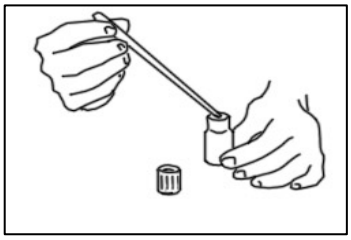
**Step 1. Opening**

- Open the vial, taking care to flip the cap so that the inner surface does not come into contact with the surface to avoid contamination.



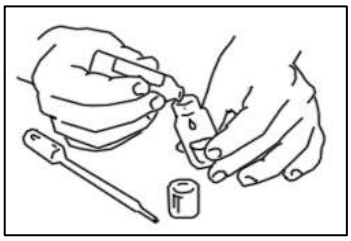
**Step 2. Inserting the sample**

- Using a pasteur dropper, sterile, disposable (supplied on request) draw a quantity of fluid to examine and introduce about 1 ml of that liquid into the vial.



**Step 3. Start analysis**

- Open the vial of water supplied with the reaction vial, and insert the entire contents of the vial itself.
- Mix by inverting the vial several times to completely dissolve the reagent (twenty seconds).
- If necessary, fill in the Quality Control Sheet.
- Place the vial in the incubator thermostat.



***Note:*** *the vial grow in 2 liquid phases: the upper one consisting of white oil transparent (does not change during the analysis), formed at the bottom of the vial from the water with which the reagent is dissolved. The color of the second phase will develop fully within 15-20 minutes from the dissolution of the reagent and that is what must be observed for the evaluation of the results of the analysis.*

**Step 4. Control of the analysis result**

- You only need to check the color of the vial once, after a set number of hours depending on the type of analysis. This number of hours does not depend only on the type of analysis, but includes the operational limit of acceptable bacterial load in the sample (the generally accepted limits for the type of sample taken by

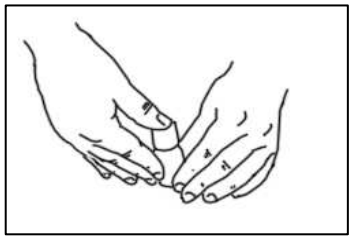
law, are shown, **by way of illustration** on the Quality Control Sheets).

- Compare the color of the vial with the correlation table set out in paragraph 1.3 or in Quality Control Sheet.
- If you have started the compilation of the Quality Control Sheet, fill it in with the result of the analysis.
- In the case of analysis for detection of *E. coli* and **only if the results are positive** (bright yellow vial), you can perform a test to confirm the presence of *E. coli*, adding 3-5 drops of Kovacs reagent to the vial at the end of the analysis, **before the sterilization**. If there is *E. coli*, within a few seconds you will see the development of a small surface layer of purple-red liquid.

***Note: to make the test for confirmation of E. coli, open the vial carefully**, avoiding contamination with the liquid contained in it, because if the color is tacked, the vial will contain a significant number of bacteria and it is therefore preferable to use disposable gloves, even non-sterile, for the operation of opening the vial. If you come in contact with the liquid, wash the affected area immediately and thoroughly with antibacterial soap or normal soap and then with a disinfectant.*

**Step 5. Post-analysis sterilization**

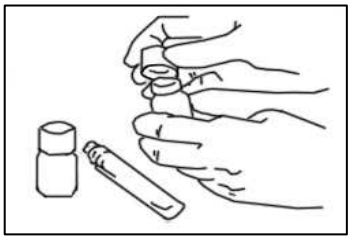
- Without opening the vial, firmly press the top of the cap and shake for about 10 seconds. The addition of the sterilizing agent can cause a color change. After 5-10 minutes the contents of the vial are completely sterilized.



***Note:** when the sterilization of the microorganisms are destroyed, the reagents are rendered inert and the vial can be safely disposed of as a "sanitary waste non-hazardous" according to the D.M. of 25/5/89. Such waste, after sterilization, can be disposed of in the same way as drugs which have expired.*

### 1.2.3 Surface analysis

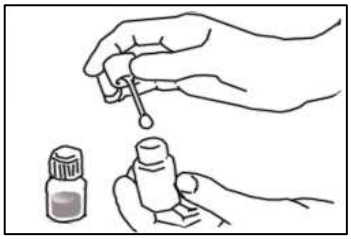
**Step 1. Preparation of the reaction vial**



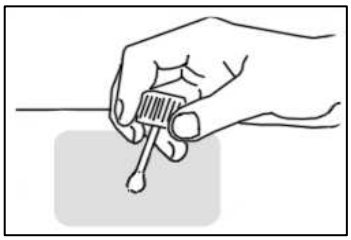
- Open the vial of water supplied with the vial for analysis, and insert the entire contents of the reaction vial itself.
- Mix by inverting the vial several times to completely dissolve the reagent (twenty seconds).

***Note:*** *after dissolution of the reagent, it is advisable to unscrew the reaction vial cap of analysis to facilitate subsequent operations.*

**Step 2. Sampling**

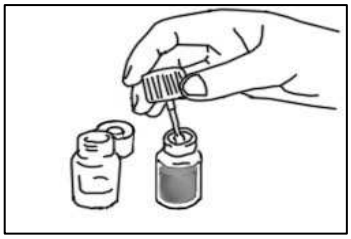


- Open the cotton swab vial containing the neutralizing solution.
- Rub the swab on the surface trying to cover a square area of about 10 cm per side.



***Note:*** *do not re-insert the swab into the vial containing the neutralizing solution.*

**Step 3. Start analysis**



- Open the previously prepared reaction vial.
- Insert the swab into reaction vial.
- If necessary, fill in the Quality Control Sheet.
- Place the vial in the incubator thermostat.

***Note:*** *you can reseal the vial containing the neutralizing solution with the cap of reaction vial before disposal.*

***Note:*** *the vial will grow in 2 liquid phases: the upper one consisting of white transparent oil (does not change during the analysis), formed at the bottom of the vial from the water with which the reagent is dissolved. The color of the second phase will develop fully within 15-20 minutes from the dissolution of the reagent and that is what must be observed for the evaluation of the results of the analysis.*

**Step 4. Control of the analysis result**

- You only need to check the color of the vial only once, after a set number of hours depending on the type of analysis. This number of hours does not depend only on the type of analysis, but includes the operational limit of acceptable bacterial load in the sample (the generally accepted limits for the type of sample taken by law, are shown, **by way of illustration** on the Quality Control Sheet)..
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**Step 5. Post-analysis sterilization**

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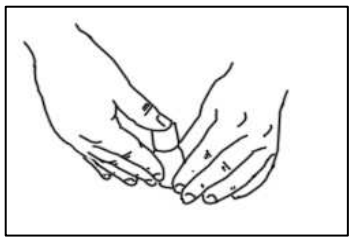

***Note:*** *with the sterilization of the microorganisms are destroyed, the reagents are rendered inert and the vial can be safely disposed of as a "sanitary waste non-hazardous" according to the D.M. of 25/5/89. Such waste, after sterilization, can be disposed of in the same way as drugs which have expired.*

## 1.3 Color scales and correlation tables

As required by law, the concentration of bacteria is expressed in CFU/g or CFU/ml or CFU/cm$^2$ (Colony Forming Units), respectively, for the analysis of solid or liquid samples or surfaces, by analogy with the results expressed with the traditional analysis, even if the MBS method is based on the detection of bacterial metabolism and not on the replication of microorganisms. The credits (approximately) correspond to individual bacterial cells.

### 1.3.1 Total Viable Count – CBT-A01

| Detection of aerobic or microaerophilic mesophilic microorganisms able to grow on complete media. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | ANALYTICAL METHOD | | | | | INCUBATION TEMPERATURE | | |
| CBT-A01 - Total Viable Count | | | MBS - MICRO BIOLOGICAL SURVEY | | | | | 30 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | POSITIVE | | NEGATIVE | |
| | | 24 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 3.30 | 3.30 | 6.30 | 9.30 | 12.30 | 16.00 | 19.00 | 22.00 | > 24.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g o CFU/ml] = $10^{(7.1 - 0.32\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(7.1 - 0.32\ x)}$ } / 100 |

### 1.3.2 Coliforms survey – CO-A02

| Rod-shaped aerobic microorganisms, Gram-negative, non spore-forming, cytochrome oxidase negative, fermenting lactose with production of acids in the presence of bile salts or other surfactants. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | ANALYTICAL METHOD | | | | | INCUBATION TEMPERATURE | | |
| CO-A02 - Coliforms | | | MBS - MICRO BIOLOGICAL SURVEY | | | | | 37 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | POSITIVE | | NEGATIVE | |
| | | 24 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 3.30 | 3.30 | 6.30 | 9.30 | 12.30 | 16.30 | 19.00 | 22.00 | > 24.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g o CFU/ml] = $10^{(7.1 - 0.32\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(7.1 - 0.32\ x)}$ } / 100 |

### 1.3.3 *E. coli* survey – CO-A02

| Rod-shaped aerobic, Gram-negative, non spore-forming, cytochrome oxidase negative microorganism; fermenting lactose with production of acids in the presence of bile salts or other surfactants; at temperature of 44 °C produce indole from tryptophan. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| CO-A02 - *E. coli* | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 44 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | 30 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 2.00 | 2.00 | 5.30 | 9.30 | 13.00 | 18.00 | 22.00 | 26.00 | > 30.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g o CFU/ml] = $10^{(6.3 - 0.24\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(6.3 - 0.24\ x)}$ } / 100 |

### 1.3.4 Enterobacteriaceae survey – EB-A04

| Gram-negative microorganisms, aerobic-anaerobic facultative, non spore-forming, oxidase negative, ferment glucose and lactose with gas production, reduce nitrate and are negative for oxidase test. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| EB-A03 - *Enterobacteriaceae* | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 37 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | 24 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 4.30 | 4.30 | 7.30 | 10.00 | 13.00 | 16.00 | 19.00 | 22.00 | > 24.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g or CFU/ml] = $10^{(7.5 - 0.34\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(7.5 - 0.34\ x)}$ } / 100 |

### 1.3.5 *Staphylococcus aureus* survey – SP-A04

| Gram-positive cocci, coagulase positive, catalase positive, fixed, non spore-forming, facultative anaerobes, fermenting mannitol and osmotolerant. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| SP-A04 - *Staphylococcus aureus* | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 37 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | 48 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 7.30 | 7.30 | 11.00 | 20.00 | 29.30 | 36.00 | 43.30 | 46.00 | > 48.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g o CFU/ml] = $10^{(6.51 - 0.13\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(6.51 - 0.13\ x)}$ } / 100 |

### 1.3.6 *Pseudomonas aeruginosa* survey – PAO-A05

| Moveable non spore-forming microorganism, rod-shaped, Gram-negative aerobic-anaerobic facultative, cytochrome oxidase and catalase positive; produce pyocyanine. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| PAO-A05 - *Pseudomonas aeruginosa* | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 37 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | 24 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 4.30 | 4.30 | 7.30 | 10.00 | 13.00 | 16.00 | 19.00 | 22.00 | > 24.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g or CFU/ml] = $10^{(7.5 - 0.34\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(7.5 - 0.34\ x)}$ } / 100 |

### 1.3.7 *Salmonella* spp. survey – SL-A06

| Gram-negative, aerobic-anaerobic facultative enterobacteria, able to ferment mannitol. Catalase positive, produce hydrogen sulfide, reduce nitrate to nitrite. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| SL-A06 - *Salmonella* spp. | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 37 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | 32 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 4.00 | < 4.00 | 4.00 | 8.30 | 13.30 | 18.00 | 23.00 | 28.00 | > 32.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g o CFU/ml] = $10^{(5.8 - 0.21\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(5.8 - 0.21\ x)}$ } / 100 |

### 1.3.8 *Listeria* spp. survey – LY-A07

| Gram-positive, non spore-forming, facultative anaerobic microorganisms, resistant to many antibiotics. Grow at pH between 5 and 9 and in the presence of NaCl to 10%. Catalase positive, oxidase negative, do not hydrolyze urea, gelatin and casein. Do not reduce nitrates, do not produce indole and hydrogen sulfide. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| LY-A07 - *Listeria* spp. | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 37 °C | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | 36 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 7.30 | 7.30 | 12.00 | 16.30 | 20.30 | 25.00 | 29.00 | 33.00 | > 36.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g o CFU/ml] = $10^{(7.7 - 0.23\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(7.7 - 0.23\ x)}$ } / 100 |

### 1.3.9 *Enterococcus faecalis* survey – EF-A09

| Gram-positive, fixed, facultative anaerobes, non hemolytic, catalase negative microorganism; fermenting glucose without gas production and hydrolyze esculin. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| EF-A09 - *Enterococcus faecalis* | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 37 °C | | |
| COLOR OF ANALYSIS AT START | | | MAXIMUM TIME OF ANALYSIS | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | | 24 HOURS | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 4.30 | 4.30 | 7.30 | 10.00 | 13.00 | 16.00 | 19.00 | 22.00 | > 24.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | $> 10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | $[CFU/g \text{ or } CFU/ml] = 10^{(7.5 - 0.34\ x)}$ |
| CONTAMINATION [CFU/cm²] | $> 10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | $[CFU/cm^2] = \{ 10^{(7.5 - 0.34\ x)} \} / 100$ |

### 1.3.10 *Aspergillus* spp. survey – AS-A10

| Multicellular eukaryotic, heterotrophic, highly aerobic organisms, growing in carbon-rich substrates, resistant to high concentrations of antibiotics and sulfonamides. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| AS-A10 - *Aspergillus* spp. | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 30 °C | | |
| COLOR OF ANALYSIS AT START | | | MAXIMUM TIME OF ANALYSIS | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | | 160 HOURS | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | 0 - 72.00 | | | | 72.00 - 150.00 | | | > 160.00 | | EQUATION |
| CONTAMINATION [CFU/g or ml] | $> 10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | - |
| CONTAMINATION [CFU/cm²] | $> 10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | - |

### 1.3.11 *Saccharomyces* spp. survey – SC-A11

| Unicellular eukaryotic, heterotrophic, highly aerobic organisms, growing in carbon-rich substrates, resistant to high concentrations of antibiotics and sulfonamides. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | |
| SC-A11 - *Saccharomyces* spp. | | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 30 °C | | |
| COLOR OF ANALYSIS AT START | | | MAXIMUM TIME OF ANALYSIS | COLOR OF ANALYSIS AT END | | | | POSITIVE | | NEGATIVE |
| | | | 60 HOURS | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 6.00 | 6.00 | 13.00 | 20.30 | 27.30 | 35.00 | 42.00 | 50.00 | > 60.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | $> 10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | $[CFU/g \text{ o } CFU/ml] = 10^{(6.8 - 0.14\ x)}$ |
| CONTAMINATION [CFU/cm²] | $> 10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | $[CFU/cm^2] = \{ 10^{(6.8 - 0.14\ x)} \} / 100$ |

## 1.3.12 *Clostridium perfringens* survey – CP-A17

| Obligate anaerobic, Gram-positive microorganism, without cytochromes, able to form spores, catalase and peroxidase negative. Can fix nitrogen, produce the cap and is mild proteolytic; makes the butyric fermentation. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ANALYSIS CODE - ANALYSIS NAME | | | ANALYTICAL METHOD | | | | INCUBATION TEMPERATURE | | | |
| CP-A17 - *Clostridium perfringens* | | | MBS - MICRO BIOLOGICAL SURVEY | | | | 44 °C | | | |
| COLOR OF ANALYSIS AT START | | MAXIMUM TIME OF ANALYSIS | | | COLOR OF ANALYSIS AT END | | POSITIVE | | NEGATIVE | |
| | | 36 HOURS | | | | | | | | |
| TIME OF COLOR CHANGE [hours.minutes] | < 7.30 | 7.30 | 12.00 | 16.30 | 20.30 | 25.00 | 29.00 | 33.00 | > 36.00 | EQUATION (x = time in hours) |
| CONTAMINATION [CFU/g or ml] | > $10^6$ | $10^6$ | $10^5$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | [CFU/g o CFU/ml] = $10^{(7.7 - 0.23\ x)}$ |
| CONTAMINATION [CFU/cm$^2$] | > $10^4$ | $10^4$ | $10^3$ | $10^2$ | 10 | 1 | 0 | | | [CFU/cm$^2$] = { $10^{(7.7 - 0.23\ x)}$ } / 100 |